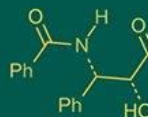


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(10): 1464-1472
www.biochemjournal.com
Received: 01-08-2024
Accepted: 05-09-2024

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Effects of zinc oxide nanoparticles on growth performance of *Cirrhinus mrigala*

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DOI: <https://doi.org/10.33545/26174693.2024.v8.i10s.2894>

Abstract

Zinc is a trace mineral which is crucial for growth, immune system, and autoxidation defence systems in fish nutrition. The goal of the present study was to determine how well various zinc sources worked as a supplement in fish feed on the growth of *Cirrhinus mrigala*. The present investigation aimed to evaluate the impact of several dietary inorganic zinc sources, including zinc oxide and zinc oxide nanoparticles, on growth performance. For 56 days, this experiment was conducted in a lab setting. A total of 225 mrigal advanced fry of uniform size were divided in triplicate among five experimental groups, with 15 fish per tank. Each tank had a completely aerated system and was filled with 120 litres of tap water. To regulate the quality of the water, one third of the water was siphoned every other day. The fish were given two different diets: a basic diet (control) and a treated diet enriched with zinc oxide at 30 mg/kg (T₁) and nanoparticles of zinc oxide at 30, 15, and 7.5 mg/kg (T₂, T₃, and T₄) in that order. Diets are given at a rate of 3% of their body weight and fed twice a day. During experiment every week the growth performance was documented. Following a 56-day feeding trial, there were notable ($p < 0.05$) variations in growth performance between the control and other treatments. Higher body weight gain, a lower FCR, and a specific growth rate were seen in treatment groups T₁, T₂, T₃, and T₄. Compared to the control and another treatment, T₃ (15 mg/kg) showed the most growth. Growth in the treatment groups surpassed the control groups. Based on the findings, it can be said that 15 mg nano ZnO/kg of supplemented feed significantly ($p < 0.005$) improved mrigal growth performance, making it a viable alternative to the control diet for improved production performance.

Keywords: Mrigal, *cirrhinus mrigala*, growth, nano ZnO, nanoparticles

Introduction

Aquaculture is an excellent option for dealing with, and even exceeding, the productivity of fishing activities while alleviating stress on natural resources (Freitas *et al.*, 2020) ^[12]. Aquaculture is the industry that produces food at the fastest rate in the world and currently supplies 52% of the fish consumed worldwide. Fish's nutritional advantages contribute to improved food security and lower rates of poverty in developing nations. Over the past ten years, intensive aquaculture has grown significantly (FAO, 2020) ^[10]. Global aquaculture production is predicted to keep expanding quickly and contribute more and more to the world's protein production (Garlock *et al.*, 2020) ^[13]. According to research, Asia accounted for 89% of the world's total fed aquaculture production in terms of quantity and 69% of its value. Around 179 million tonnes (MT) of edible fish were reported to have been supplied to the world in 2018 by aquaculture and fishing, translating to a per capita supply of 20.5 kg (live weight equivalent). 82 MT came from aquaculture output, and the fish's total initial marketplace value was USD 401 billion (FAO, 2020) ^[10]. As one of the most important food production systems in terms of economic effect and food security, aquaculture's sustained expansion is crucial to the strategy for guaranteeing the nutritional safety of the global population.

Dietary minerals known as trace elements are needed in very small amounts for regular physiological processes. They mostly serve as cofactors or structural elements of enzymes, and their functions include immune response, antioxidant defence, gene expression regulation, immune prophylaxis, and the avoidance of nutritional deficits. Zinc is the second most prevalent trace element found in the animal body, following iron. It can be taken regularly to satisfy the body's physiological needs, but the body is unable to store it. (Zalewski *et al.*, 2005) ^[39].

Numerous functions in animals depend on it, including growth, metabolism, immunological response, wound healing, fertilization, and the maintenance of oxidative stress (Kietzmann and Braun, 2006^[17]; Feng *et al.*, 2010^[11]; Liu *et al.*, 2011^[21]; Zhao *et al.*, 2014^[40]; Kisku *et al.*, 2024)^[19]. Due to its involvement in numerous metabolic pathways, zinc is a crucial trace element in fish nutrition. Zinc is a vital trace element for finfish and is necessary for over 300 enzymes and vitamins to operate, as well as for biological processes and physiological activities include hormone production, enzymatic activity, and protein, carbohydrate, and lipid metabolism. (Sau *et al.*, 2004^[31]; Sau and Paul, 2004^[31]; Muralisankar *et al.*, 2014^[26]; Bera *et al.*, 2022a^[1]; Bera *et al.*, 2022b^[2]; Karjee *et al.*, 2023 and Kisku *et al.*, 2024)^[19, 16]. The physiology, growth, and development of animals depend on about a thousand structural, catalytic, and regulatory proteins (Eide, 2006^[8]; Maret and Krezel, 2007)^[23].

Nanotechnology, whose name comes from the Latin word "nanus," which means "dwarf," is the study of materials and structures whose sizes are measured in nanometers. The word "nano" more accurately refers to 10^{-9} or one billionth of a meter. Materials between 1 and 100 nm in size are referred as nanomaterials (Rai and Ingle, 2012)^[28]. Now a days, many nanotechnology-based technologies are being used now to boost sustainability, efficiency, and productivity (Carlos *et al.*, 2022)^[4]. Aquaculture systems could undergo significant improvements because to nanotechnology, which could reduce costs, increase productivity, and diminish our environmental impact. When it comes to the rapid development and use of nanotechnology in the agri-food sector, nanoparticles (NP) have great promise for enhancing immunity, controlling infections, and promoting aquaculture growth (Brintha and Ajitha, 2015)^[3]. Zinc oxide, one of the many metal oxide nanoparticles, is essential to the field of nanotechnology because it is more specific than other micro and nano metal oxides. It is also given to water and food as a vitamin to support plankton production and as a source of zinc for fish growth. Research has already demonstrated that ZnO NP has a dose-dependent influence on livestock and poultry growth performance (Hongfu, 2008^[15]; Yang and Sun, 2006^[38]; Lina *et al.*, 2009^[20]; Mishra *et al.*, 2014^[24]; Sahoo *et al.*, 2014)^[30].

The semi-intensive culture of Indian major carps has seen significant success in the standardization and indigenous development of various aquaculture artifacts. However, a major obstacle to the more intensive culture of these fishes is the lack of appropriate feed development, which necessitates a precise understanding of the nutritional requirements of the fish species. Natural feeds like planktons are also important source of natural nutrition and these can be natural food to the fish when cultured in pond or other closed natural systems (Das *et al.*, 2013a; Das *et al.*, 2013b; Sau *et al.*, 2012; Sau *et al.*, 2013; Das *et al.*, 2014)^[5, 6, 7, 33, 34]. However, the effects ZnO NP on haematological responses of *Cirrhinus mrigala* in the agro-climatic condition of West Bengal, India was assessed by Kisku *et al.* (2024)^[19]. Regarding the mineral requirements of the Indian major carps, *Cirrhinus mrigala*, not much is known, but this knowledge is crucial for feed compositions. Therefore, we investigated the effect of several zinc sources, such as zinc nanoparticles (ZnO NP), as feed additive in diets to increase growth performance of *Cirrhinus mrigala*.

Materials and Methods

The study was conducted with 15 tanks of 243 litre capacity. The tanks were cleaned thoroughly using scrub and then sun-dried for three days. Tanks were placed in triplicate and then they were filled with tap water up to 162 litres maintaining 30 cm height and providing optimal aeration throughout the experiment.

Stocking of experimental fish

Healthy and uniform mrigal (*Cirrhinus mrigala*) fingerlings irrespective of sex were used for all the experiments. Three hundred (300) fish were collected from Naihati fish seed hatchery, Kolkata, West Bengal. Fish were brought to the laboratory with proper oxygen packaging and on reaching the laboratory, the experimental fishes were disinfected by immersion in 0.02 ppm potassium permanganate (KMnO₄) solution for 10 min, then released into the glass tanks. Keeping them unfed for 24 hours, then they are fed with sinking palletted control feed (28.37% protein) for 7 days @ 3% body weight with proper aeration facilities where they got acclimatized fully before experimentation. Chlorine-free aerated tap water was used throughout the experiment. Faecal matters were siphoned out and one-third of the water exchange was done with fresh water on every alternative day. During the acclimatization period, the fish showed no abnormalities such as lethargy, opercular flaring, abrupt swimming, etc.

Experimental design

Two hundred twenty-five (225) fish with average body weight (3.63 ± 0.01 g) were allotted into five experimental groups. Each group is subdivided into three replicates and each replicate comprises fifteen (15) fish selected at random. Each fish's body weight was measured, and the mean body weight of the experimental groups was maintained identically throughout the allocation process.

Fish and Rearing Conditions

Following acclimatization, 225 fish with a mean body weight of (3.63 ± 0.01 g) were stocked in 15 aquaria tanks in triplicate, at a number of 15 fish per tank. Each fish's body weight was measured, and the mean body weight of the fish remained constant ($p > 0.05$) during the allocation period. Fish were kept from jumping out of the tanks by covering them with nylon netting as well as to avoid insects and dust introduction. Feeding was done two time a day (9 AM and 4 PM) at satiation level. The excess feed and faecal matters were siphoned daily before and after feeding. The physicochemical characteristics of the water were measured periodically to maintain the optimal level throughout the experiment.

Preparation of feed

The raw materials are chosen according to their capacity to provide nutrients. As a protein source, groundnut oil cake and soybean meal were utilized; using wheat flour as a source of carbohydrates; as fat sources, fish oil and sunflower oils were employed. The different supplements were also added to make the feed balanced. The feed ingredient materials were ground into powder, dried, and then sieved through a 425-micron sieve. Next, the Micro-Kjeldahl method was used to determine the protein content of the key ingredients. The ingredient materials were then carefully blended with 130-150 ml of distilled water after

being weighed. After being autoclaved for 15 minutes at 100 °C, the mixture of feedstuff was cooled. After cooling, fish oil, sunflower oil, lecithin, vitamin mixtures and binders were mixed with the ingredients, and different concentrations of nano zinc oxide were added as required in the treatment feed. The control feed was prepared without any zinc source. The treatment feeds were prepared with standard ingredients along with normal zinc of 30 mg/kg (T₁), nano zinc 30 mg/kg (T₂), nano zinc 15 mg/kg (T₃), and nano zinc 7.5 mg/kg (T₄). Then the pellets were made and allowed to dry at room temperature with the use of a manual pelletizer machine. To avoid contamination, the prepared meal was stored at -20 °C in an airtight container until it was needed. The diet composition was as per Kisku *et al.* (2024) [19].

Growth performance

A standard protocol has been used to calculate the weekly body weight, average body weight gain (ABWG), daily weight gain (DWG), specific growth rate (SGR), food conversion ratio (FCR) of the experimental fish (*Cirrhinus*

mrigala).

Body weight

The fish in various treatment tanks had varying growth patterns, and the fish's body weight generally followed an expected temporal trend of increasing over time. Throughout the experiment, there were variations in the growth rate in different weeks. The T₃ group had the highest average body weight of 21.62 g, whereas the control group had the lowest average weight of 15.33 g. There was a significant difference ($p<0.05$) according to the ANOVA analysis. From the table-1, it was evident that *Cirrhinus mrigala* had weekly growth exhibited a substantial increase for all treatments, including control ($p<0.05$). But there were variations of growth obtained within the treatments. The T₁, T₂, and T₄ treatments did not significantly change throughout the first week. But compared to other groups, T₃ shown a notable change. The same type of relation among the treatments and control group were found at 5th, 6th, 7th and 8th week also. When comparing the control and treatment tanks, the control group's growth rate was slower every week.

Table 1: The weekly growth (g) of *Cirrhinus mrigala* treatments wise.

Weeks	C	T ₁	T ₂	T ₃	T ₄
Initial	3.61±0.06 ^A	3.63±0.06 ^A	3.63±0.08 ^A	3.64±0.03 ^A	3.62±0.05 ^A
1st	4.83±0.07 ^{aB}	5.22±0.12 ^{bB}	5.29±0.05 ^{bB}	5.71±0.19 ^{cB}	5.14±0.04 ^{bB}
2 nd	6.18±0.07 ^{aC}	6.77±0.09 ^{bC}	6.95±0.05 ^{cC}	7.67±0.16 ^{dC}	6.60±0.07 ^{bC}
3 rd	7.67±0.04 ^{aD}	8.44±0.08 ^{cD}	8.68±0.05 ^{dD}	9.71±0.25 ^{eD}	8.21±0.08 ^{bD}
4 th	9.22±0.04 ^{aE}	10.17±0.12 ^{cE}	10.41±0.07 ^{dE}	11.87±0.18 ^{eE}	9.85±0.11 ^{bE}
5 th	10.74±0.05 ^{aF}	11.90±0.09 ^{bF}	12.16±0.08 ^{bF}	14.66±0.19 ^{cF}	11.59±0.08 ^{bF}
6 th	12.26±0.20 ^{aG}	13.67±0.14 ^{bG}	13.94±0.13 ^{bG}	16.77±1.10 ^{cG}	13.39±0.07 ^{bG}
7 th	13.79±0.40 ^{aH}	15.43±0.16 ^{bH}	15.78±0.10 ^{bH}	18.97±1.27 ^{cH}	15.11±0.09 ^{bH}
8 th	15.33±0.63 ^{aI}	17.41±0.38 ^{bI}	17.95±0.47 ^{bI}	21.62±1.22 ^{cI}	16.84±0.18 ^{bI}

*Data presented in mean ±SD. Capital superscripts showing significance level column-wise ($p<0.05$), small superscripts showing significant level row-wise ($p<0.05$). There was noticeable growth changes obtained by all groups at 3rd and 4th week. However, there was no significant difference of growth obtained within T₁ and T₄ at 2nd week.

The temporal variation of the average body weight of fish in the system is presented in figure- 1. The Relationship of body weight (g) week-wise of different treatment groups of *Cirrhinus mrigala* (C, T₁, T₂, T₃, T₄) was presented in figure 2.

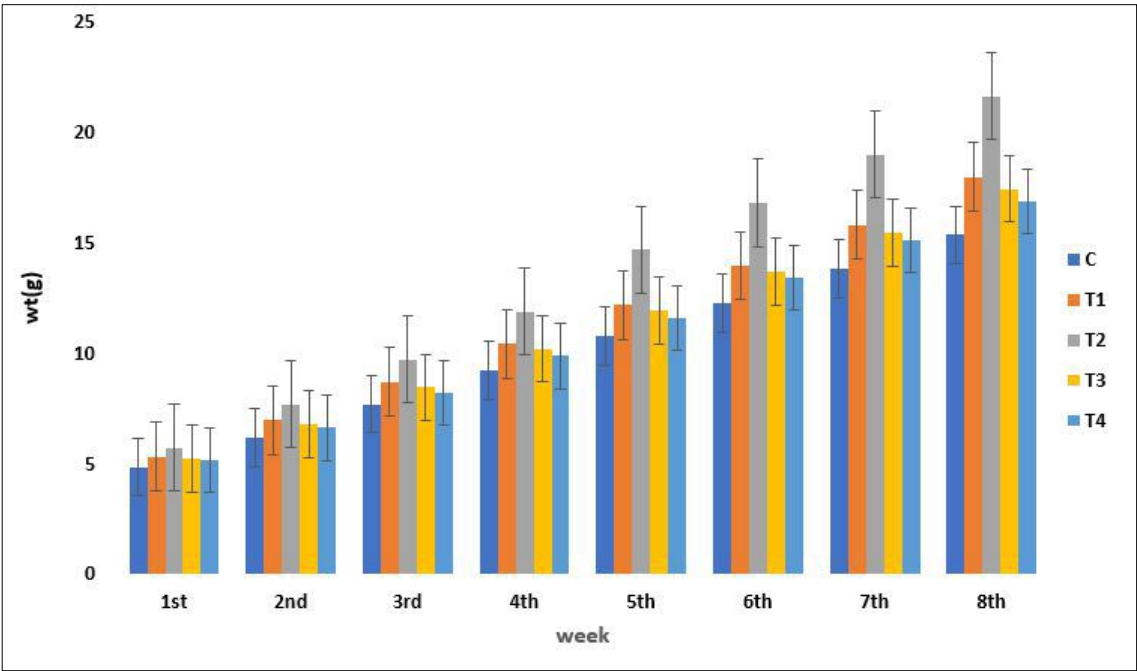
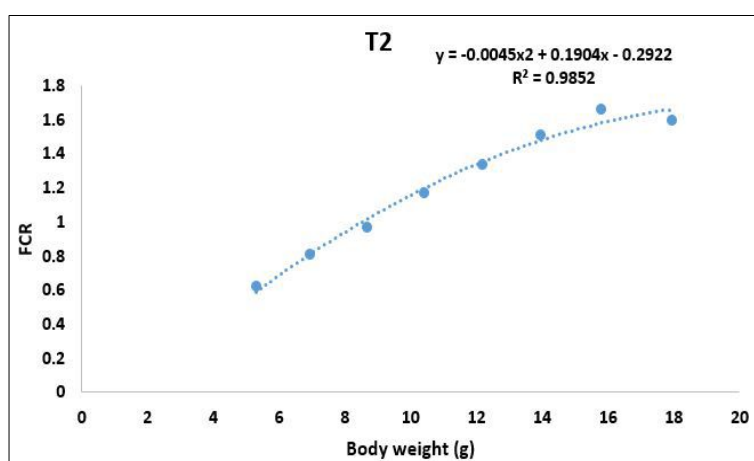
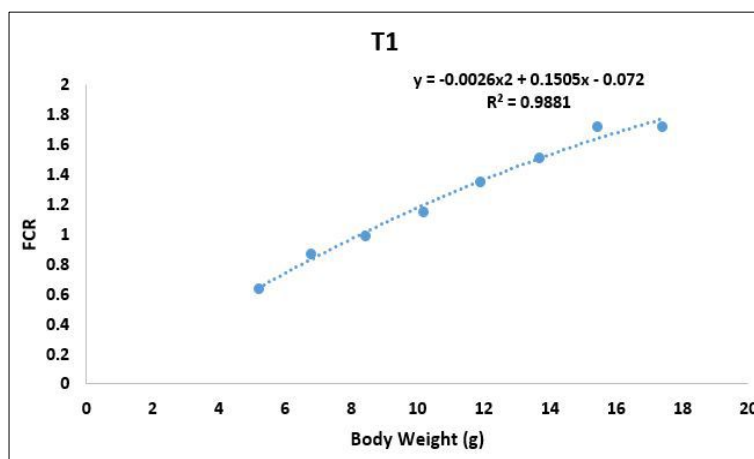
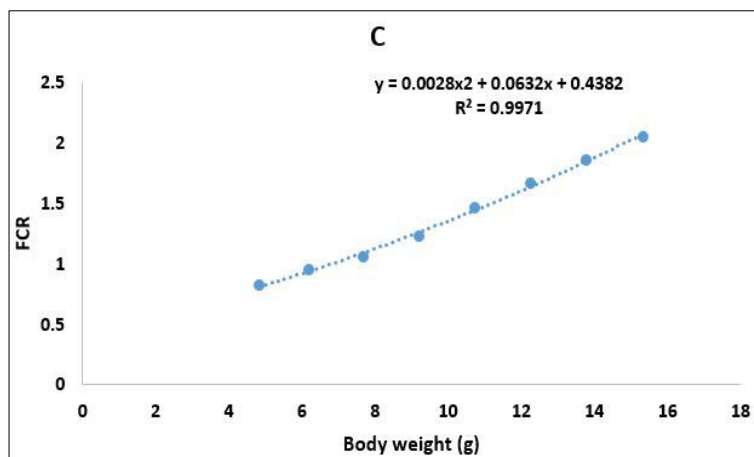


Fig 1: Weekly variation of average body weight of *Cirrhinus mrigala*

Similar to this work, Faiz *et al.* (2015) ^[9] found that after the trial, the change in body weight was significant between the Zn-supplemented diet and the basal diet in grass carp. Also, Mondal *et al.* (2020) ^[25], studied that rohu fish fed with ZnO nanoparticles enriched feed were able to attain a higher body weight than fish on a basic diet. Among various treatments, the diet containing 20 mg/kg of ZnO was shown to yield the highest body weight. In this investigation, feed

treated with 15 mg/kg of nZnO showed higher body weight gain than other groups. But according to Mahboub *et al.* (2020) ^[22], nZnO 20 mg/kg and nZnO 30 mg/kg supplemented meals gained the highest final body weight in African catfish. Swain *et al.*, (2019) ^[36] also reported that body weight increased higher in nano zinc and nano selenium supplemented diet as compared to basal diet.



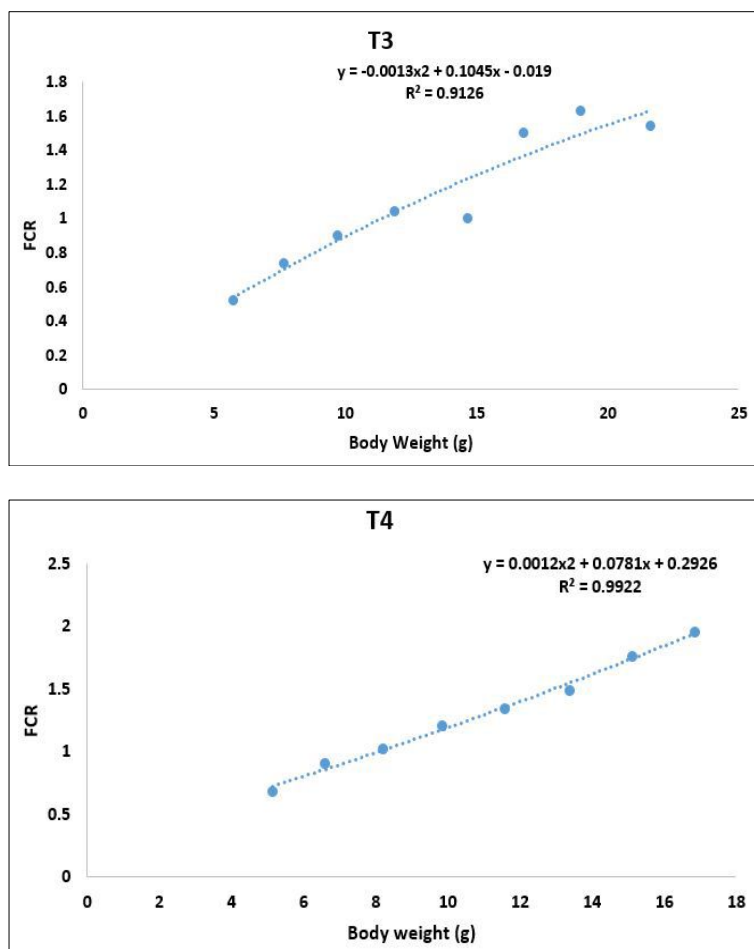


Fig 2: The relationship of body weight with week-wise of *Cirrhinus mrigala* under control and different treatments.

Average body weight gain (%)

The average body weight gain (ABWG) reached by the experimental fish was documented on a weekly basis in the current study, where weight growth rose as the investigation went on. On the basis of it, we can observe that all fish, with the exception of the T₃ treatment fish, reached optimal ABWG. The T₃ treatment had the highest ABWG of 17.93±1.20 (g), whereas the control tank had the lowest ABWG of 11.65±0.65 g. The T₃ tank reached its maximum peak between the fourth and fifth week, after which it declined and then grew once more between the sixth and eighth weeks. The ABWG did not show significant

differences within T₁ and T₄ but, there was significant variances between the groups ($p < 0.05$) (Table-2).

Table 2: Average body weight gain (ABWG), daily weight gain (DWG), feed conversion ratio (FCR) and specific growth rate (SGR) of *Cirrhinus mrigala*.

Treatment	ABWG	DWG	SGR	FCR
C	11.65±0.65 ^a	0.208±0.01 ^a	2.58±0.003 ^a	2.06±0.01 ^d
T ₁	13.76±0.33 ^b	0.245±0.01 ^c	2.8±0.00 ^b	1.72±0.01 ^b
T ₂	14.26±0.57 ^c	0.254±0.01 ^c	2.85±0.01 ^b	1.60±0.02 ^b
T ₃	17.93±1.20 ^d	0.320±0.02 ^d	3.18±0.01 ^c	1.54±0.01 ^a
T ₄	13.24±0.05 ^b	0.236±0.01 ^b	2.74±0.02 ^b	1.96±0.02 ^c

*Data presented in mean ±SD. Small superscripts showing significance level column-wise ($p < 0.05$).

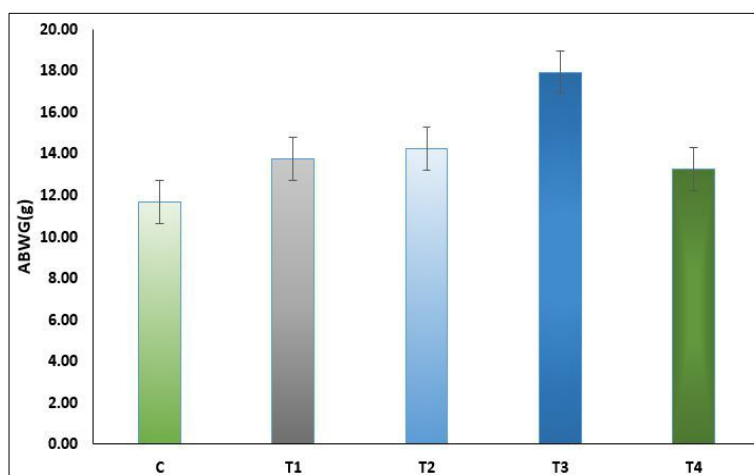


Fig 3: Variation of total average body weight gain of *Cirrhinus mrigala*

The average trend of body weight gain was slightly increasing, but the T₃ group showed a significantly increased trend of weekly weight gain in the fifth week (figure-3). In comparison to other diets with ZnO concentrations of 30 mg/kg and nZn concentrations of 7.5 and 30 mg/kg, the current investigation on mrigal shown that higher weight gain is attained with 15 mg/kg of nZnO. In a similar study, Rajan and Rohini (2021) [29] found that supplemented feed containing 15 mg/kg of nZnO gained greater weight than any other concentration of 5, 10, 20, or 25 gm/kg. This finding supports the results of my experiment. Ghazi *et al.* (2021) [14] report that tilapia fed a 10 mg/kg diet ZnO-NP gained more weight than those fed a control diet. Based on the research on grey mullet, Shukry *et al.* (2022) [35] found that fish feed enriched with 20-40 mg/kg of nano ZnO nanoparticles gained more weight than the control group. However, the current study found that a diet containing 15 mg/kg of ZnO nanoparticles caused more weight gain. But in catfish, ZnO 30 mg/kg nanoparticle diet showed considerably ($p < 0.05$) greater weight gain, according to Onuegbu *et al.* (2018) [27]. According to Tawfik *et al.* (2017) [37], tilapia weight gain was progressively increased by supplementing with nano zinc oxide rather than conventional zinc oxide. Nano zinc significantly increased weight gain compared to organic zinc, according to Kishawy *et al.* (2020) [18].

Daily weight gain (DWG)

According to Onuegbu *et al.* (2018) [27], catfish fed diet enhanced with nanoparticles showed a greater daily weight gain. Over the course of the study's 56-day experiment, daily weight increase was noted and treatment-wise documentation was made. The results of this investigation indicate that the control tank exhibits the lowest DWG and the T₃ treatment the highest DWG. The highest DWG is 0.32 g, while the lowest is 0.2 g. The DWG for tanks T₁, T₂, and T₄ was 0.25 g, 0.26 g, and 0.24 g, in that order. However, according to the investigation's findings, the treatment and control groups did not differ significantly ($p > 0.05$). Daily weight gain is represented in figure-4.

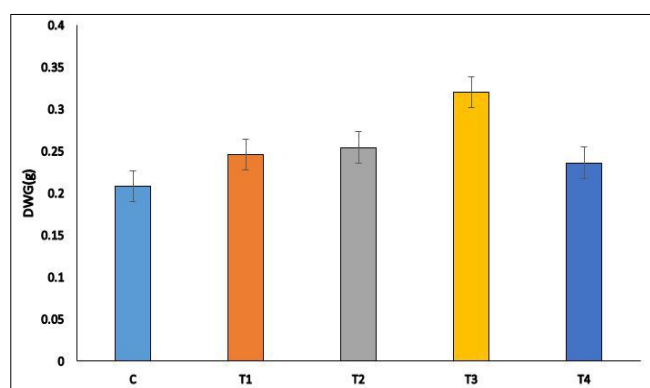


Fig 4: Treatment wise variation of DWG of *Cirrhinus mrigala*

Specific growth rate (SGR)

The SGR value of different treatment groups are shown in figure 5. The experimental fish's specific growth rate result revealed a substantial ($p < 0.005$) variation between treatment groups. T₃ had the highest SGR value (3.181 g), which was notably different from the values for the other treatments. The Control group had the lowest SGR, which was 2.582 g. The treatment groups of T₁, T₂, and T₄ did not

reveal significant changes ($p > 0.05$) shown in (figure-13). When compared to the other three treatments and the control group, T₃ showed a substantial difference. The current investigation shows that T₃ with 15 mg/kg of nZnO achieved increased SGR in Mrigal. Tawfik *et al.* (2017) [37] conducted a study on *Oreochromis niloticus* in which they fed fish with various concentrations of zinc oxide supplemented feed for 120 days at bulk scale (ZnO) and nanoscale (nZnO) at the rate of 15, 30, 45, and 60 mg/kg of the feed. Similarly greater concentrations of bulk ZnO (60 mg/kg), nZnO (15 mg/kg) produced higher specific growth rates.

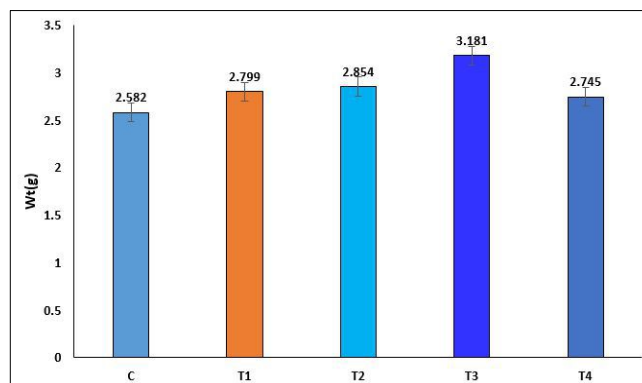


Fig 5: Treatment wise variation of SGR of *Cirrhinus mrigala*

In another study set up in pond culture for a 120-day trial, Swain *et al.*, (2019) [36] revealed that *L. rohita* in control diets grew within the normal range, but the treated diet containing Zn and Se showed faster SGR than the control group.

Feed conversion ratio (FCR)

T₃ had a lower FCR (1.54 ± 0.01) compared to the other groups, which was statistically significant ($p < 0.05$). The control group had the highest FCR (2.06 ± 0.01). There was no significant difference between T₁ (1.72 ± 0.01) and T₂ (1.60 ± 0.02). But T₄ (1.96 ± 0.01) showed a significant change when compared to the other treatments. Figure- 6 shows the FCR for the various treatment groups.

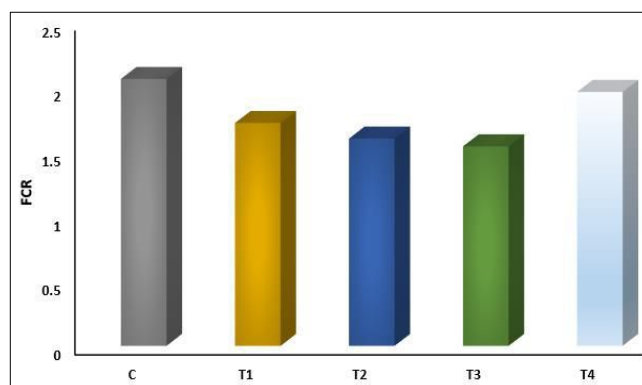
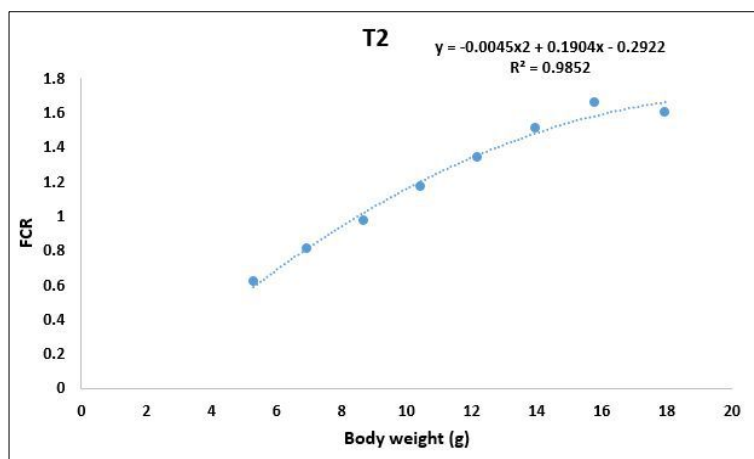
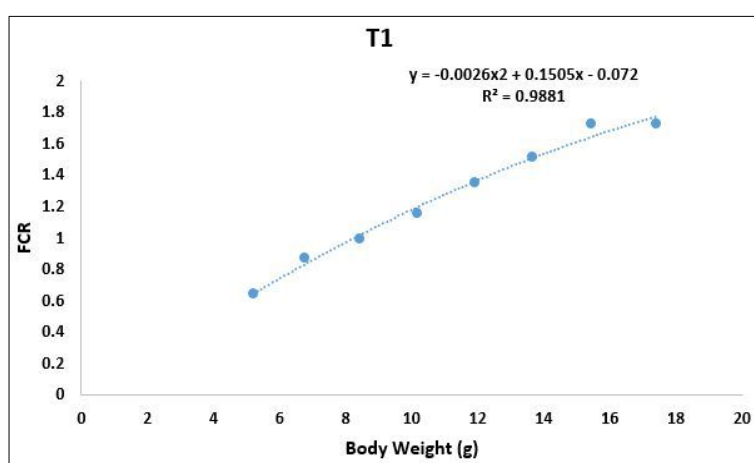
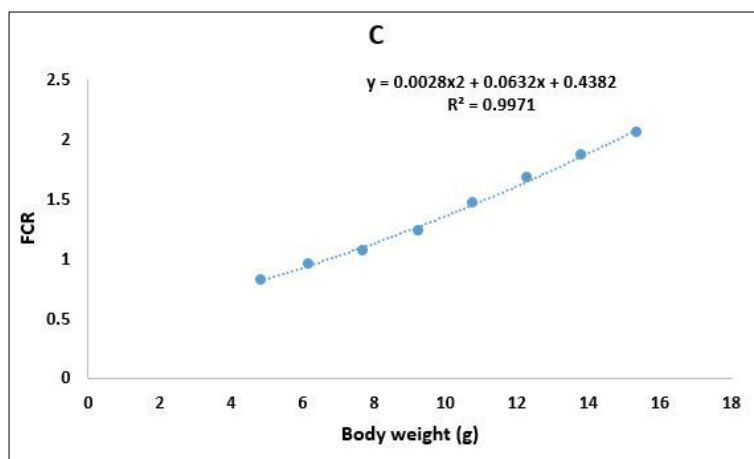


Fig 6: Variation of feed conversion ratio of *Cirrhinus mrigala*

Based on the current investigation, low FCR was achieved using T₃ treatment that included a 15 mg/kg concentration feed of nZnO. In a similar study, Mondal *et al.* (2020) [25] found that rohu had low FCR at the dose of 20 mg/kg of nZnO. According to research by Faiz *et al.* (2015) [9], juvenile grass carp fed containing higher dose of nano ZnO had a high feed conversion ratio. The current study reveals

feeding with 15 mg of nano zinc oxide increased Mrigal's feed conversion efficiency. Onuegbu *et al.* (2018) [27] also observed an increase in zinc oxide nanoparticle content decreases the FCR. The relationship between body weight

and FCR in mrigal in control and different treatments shown in figure-7. It was found that there was very strong relationship between FCR and body weight at different treatment groups along with control as R^2 is nearer to 1.



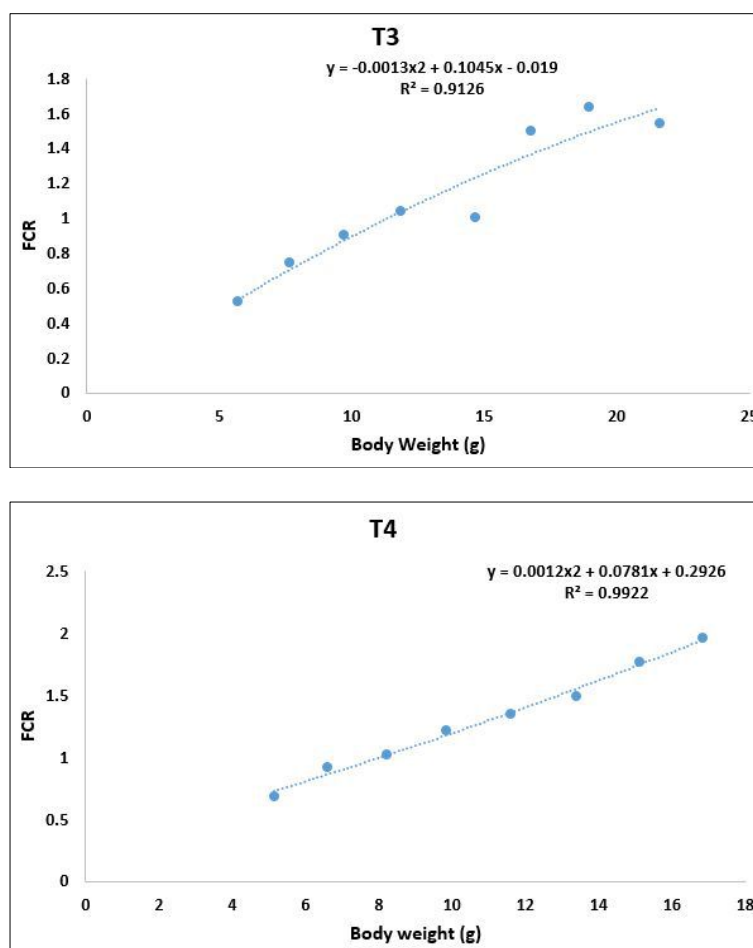


Fig 7: Relationship between body weight (g) and FCR in mrigal in control and different treatments.

Conclusion

The findings of the research will be helpful in understanding the zinc supplementation effect on mrigal growth performance and nano zinc concentration of 15mg/kg in mrigal feed will show the best performance in respect of growth and FCR. So, it is recommended to incorporate the said dose while preparing the feed for mrigal. The findings of present study reveal that zinc oxide nano form having the potential of increasing the production performance of mrigal which can help in achieving the animal nutritional demand and eliminate the global hunger problem.

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